

**Docket 85504SLP**  
**Customer No. 70523**  
**Confirmation No. 9183**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**  
**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

In re Application of

Tiecheng A. Qiao, et al.

COLORABLE MICROSPHERES  
FOR DNA AND PROTEIN  
MICROARRAY

Serial No. 10/625,424

Filed 23 July 2003

Group Art Unit: 1743

Examiner: Paul Sang Hwa Hyun

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Sir:

**APPEAL BRIEF PURSUANT TO 37 C.F.R. 41.37 and 35 U.S.C. 134**

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## **Table Of Contents**

<u>Table Of Contents</u> .....	i
<u>Real Party In Interest</u> .....	1
<u>Related Appeals And Interferences</u> .....	1
<u>Status Of The Claims</u> .....	1
<u>Status Of Amendments</u> .....	1
<u>Summary of Claimed Subject Matter</u> .....	1
<u>Grounds of Rejection to be Reviewed on Appeal</u> .....	2
<u>Arguments</u> .....	3
<u>Summary</u> .....	12
<u>Conclusion</u> .....	13
<u>Appendix I - Claims on Appeal</u> .....	14
<u>Appendix II - Evidence</u> .....	17
<u>Appendix III – Related Proceedings</u> .....	18

## **APPELLANT'S BRIEF ON APPEAL**

Appellants hereby appeal to the Board of Patent Appeals and Interferences from the Examiner's Final Rejection of claims 1-17 which was contained in the Office Action mailed 04/06/2007.

A timely Notice of Appeal was filed 10/01/2007.

### **Real Party In Interest**

Carestream Health, Inc. is the real party in interest.

### **Related Appeals And Interferences**

No appeals or interferences are known which will directly affect or be directly affected by or have bearing on the Board's decision in the pending appeal.

### **Status Of The Claims**

Claims 1-19 are pending in the application.

Claims 18 and 19 stand withdrawn from consideration as directed to a non-elected invention, pursuant to a restriction requirement made by the Examiner in an Office Action dated 12/16/2005, and the Applicant's election made in the Response dated 04/17/2006.

Claims 1-17 are being appealed.

Appendix I provides a clean, double spaced copy of the claims on appeal.

### **Status Of Amendments**

An Amendment After Non-final, including only remarks, was filed on 01/17/2007. A Final Office Action dated 04/06/2007 was then received indicating that the Examiner considered, but did not find the remarks sufficiently persuasive to place the Application in condition for allowance.

### **Summary of Claimed Subject Matter**

The invention relates to a biological microarray technology (page 1, lines 15-16). In particular, the invention relates to an array of microspheres immobilized on a substrate (page 1, lines 16-18). The microspheres contain a

colorless (page 11, line 31 to page 12 line 1) non-fluorescent (page 9, lines 11-12) latent colorant that when developed can identify the microsphere (page 4, lines 2-3). The microspheres contain biological capture probes on their surface (page 4, line 1).

Independent claim 1 recites a microarray (page 3, lines 28-30) comprising a support (page 3, line 31) having a layer of microspheres disposed thereon (page 3, line 31 to page 4, line 1). The microspheres bearing biological probes (page 4, line 1); wherein the microspheres comprise at least one colorless (page 11, line 31 to page 12 line 1) and non-fluorescent (page 9, lines 11-12) latent colorant that can be developed to a colored state and used to identify said microsphere (page 4, lines 2-3).

#### **Grounds of Rejection to be Reviewed on Appeal**

The following issues are presented for review by the Board of Patent Appeals and Interferences:

1. Whether claims 1-5, 7-12 and 14-17 are unpatentable under 35 U.S.C. § 103(a) over Chee et al. (U.S. 6,429,027) in view of Leblans et al. (U.S. 2004/0069857 A1).
2. Whether claim 13 is unpatentable under 35 U.S.C. § 103(a) over Chee et al. (U.S. 6,429,027) in view of Leblans et al. (U.S. 2004/0069857 A1), and further in view of Wang (U.S. 4,663,277).
3. Whether claims 1, 3, 5 and 6 are unpatentable under 35 U.S.C. § 103(a) over Chee et al. (U.S. 6,429,027) in view of Litt (U.S. 4,092,408).

## **Arguments**

### **Rejection of claims 1-5, 7-12 and 14-17 under 35 U.S.C. § 103(a) over Chee et al. in view of Leblans et al.:**

In the Final Office Action dated 04/06/2007 claims 1-5, 7-12, and 14-17 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Chee et al. (US 6,429,027 131) in view of Leblans et al. (US 2004/0069857 A1).

The Examiner indicates that Chee et al. discloses a two-dimensional array of microspheres randomly immobilized in wells of a substrate, wherein the microspheres bear biological probes in the form of a bioactive agent that binds an analyte of interest. The Examiner states that the microspheres can comprise a dye in the form of chromophores that can be developed to produce a unique optical signature that allows one to visually identify the microspheres and the bioactive agent bound to the microspheres, and that chromophores as defined by the specification absorb light and convert the absorbed light into heat, which is a photo initiated process. The Examiner further indicates that the microspheres disclosed by Chee et al. differ from the claimed invention in that the reference does not disclose that the dye is a colorless dye that can be developed to a colored state. The Examiner relies on Leblans et al. to teach this limitation. This rejection is respectfully urged as in error and reversal is requested for the following reasons.

Chee et al. discloses sensor compositions comprising a composite array of individual arrays, to allow for simultaneous processing of a number of samples. The compositions comprise a substrate with a surface having a plurality of assay locations, each assay location comprising an array location, the array location comprising a plurality of discrete sites; and a population of microspheres comprising at least a first and a second subpopulation, wherein the first subpopulation comprises a first bioactive agent and wherein the second subpopulation comprises a second bioactive agent. The microspheres are distributed in the discrete sites in the array location.

Leblans et al. discloses manipulating a microcarrier for identification purposes that utilizes a positioning and orienting step prior to or during the

identification step. During the identification step the encoded microcarrier can be identified and labeled.

The present invention relates to a microarray comprising a support, on which is disposed a layer of microspheres bearing biological probes, wherein the microspheres comprise at least one material with a non-fluorescent latent colorant that can be developed to a colored state and used to identify the microsphere.

To establish a prima facie case of obviousness, there must be some suggestion or motivation in the reference or in the general knowledge available to one skilled in the art to modify the reference, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all the claim limitations.

**The references fail to disclose a colorless and non-fluorescent molecule:**

Chee et al. discloses a colored molecule that changes to a different color or fluoresces. Chee et al. describes “a molecule whose color or luminescence properties change in the presence of a selectively-binding DBL.”(col. 15, lines 36-38), “a molecule whose color or luminescence properties change in the presence of various solvents.” (col. 15, lines 43-46), “a derivative of fluorescein whose color changes between aqueous and nonpolar solvents.” (col. 15, lines 48-50), and “In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic, electrical, thermal; and c) colored or luminescent dyes; although labels include enzymes and particles such as magnetic particles as well.” (col. 19, lines 33-37). Chee et al. describes a colored molecule that changes to a different color or fluoresces. Chee et al. fails to disclose a colorless and non-fluorescent molecule as claimed by the instant invention.

Leblans et al. also fails to disclose a colorless, latent, non-fluorescent colorant as presently claimed. Leblans et al. discloses the use of fluorescent materials throughout the reference. “Preferred bleachable substances include bleachable fluorescent or electromagnetic radiation absorbing substances.” (par. 0052). The reference also discloses in paragraph 0052 that suitable compounds include fluorescers, formaldehyde induced fluorescence, Flazo Orange, Fluo 3, Fluorescamine, and fluorescein isothiocyanate all of which are fluorescing.

“Optionally such bleachable substances will contain functional groups capable of forming a stable fluorescent product with functional groups typically found in biomolecules or polymers.” (par. 0052). “Codes bleached on microcarriers may also be written to have different intensities of fluorescence” (par. 0053).

Paragraphs 0159 through 0168 list examples of fluorescent microcarriers. Clearly Leblans et al. fails to teach or suggest any advantages in the use of colorless and non-fluorescent colorants as claimed by the instant invention. In fact, the reference teaches that the use of fluorescent compounds are suitable, distinguishable from the non-fluorescent colorant of the instant invention. Neither reference alone or in combination discloses a colorless and non-fluorescent latent colorant as claimed by the instant invention.

**The instant invention has surprising results:**

The microsphere of the present invention overcome the problem associated with “spectrally addressed microspheres,” colored compounds typically used in microspheres are often fluorescent, and hence provide excessive background noise when fluorimetric determinations are performed on the microarray. This problem is overcome through the use of latent colorants, which are colorless and non-fluorescent until switched to a colored state by a chemical reaction, a physical trigger, or some kind of environmental stimulus. A further surprising result of the present invention is that the colorless coding offers no detectable background fluorescence from microspheres, therefore detection is dramatically improved. As such, the dynamic range for measuring target analytes is greatly increased over the prior art.

Since the references, alone or in combination, fail to disclose all of the presently claimed limitations, the Applicants respectfully request reversal of this rejection.

**Rejection of claim 13 under 35 U.S.C. § 103(a) over Chee et al.  
in view of Leblans et al. and further in view of Wang:**

In the Final Office Action dated 04/06/2007 claim 13 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Chee et al. in view of Leblans et al. as applied to claims 1-5, 7-12, 14-17, and further in view of Wang (US 4,663,277).

The Examiner indicates that neither Chee et al. nor Leblans et al. discloses the immobilization of the microspheres by a gelation process. The Examiner further indicates that Wang discloses an immunoassay for a virus accomplished by utilizing microspheres coated with antiviral antibodies. The reference discloses that the method of the immunoassay involves immobilizing the microspheres by placing the microspheres in a gel. The Examiner states that it would have been obvious to one of ordinary skill in the art to further immobilize the modified microspheres disclosed by Chee et al. and Leblans et al. by means of a gel as taught by Wang so that the microspheres are better secured within the wells of the substrate. Reversal of this rejection is requested for the following reasons.

Chee et al. discloses sensor compositions comprising a composite array of individual arrays, to allow for simultaneous processing of a number of samples. Comprising a substrate with a surface having a plurality of assay locations, each assay location comprising an array location, said array location comprising a plurality of discrete sites; and a population of microspheres comprising at least a first and a second subpopulation, wherein said first subpopulation comprises a first bioactive agent and wherein said second subpopulation comprises a second bioactive agent; wherein said microspheres are distributed in said discrete sites in said array location.

Leblans et al. related to manipulating a microcarrier for identification purposes that utilizes a positioning and orienting step prior to or during an identification step. During the identification step the encoded microcarrier can be identified and labeled.

Wang relates to the detection and/or the determination of viruses by an immunoassay method, to materials for such method, and to a virus detection kit. Viruses are detected by means of an immunoassay method in which an extended



solid phase coated with antiviral antibody is employed to bind and remove virions from a specimen by forming an immuno-complex with antigens of the virions, a mobile solid phase comprising a dispersion of microspheres coated with the antiviral antibody is used to bind the microspheres to antigens associated with the immuno-complex, and the presence of bound microspheres is detected. The detection sensitivity is amplified by the ability to more readily detect the microspheres, which may be dyed or labeled. The extended solid phase advantageously may be in the form of a dipstick which can be easily contacted with the specimen. A virus detection kit provides the extended solid phase and mobile solid phases, each coated with antiviral antibodies.

The present invention relates to a microarray comprising a support, on which is disposed a layer of microspheres bearing biological probes, wherein the microspheres comprise at least one material with a non-fluorescent latent colorant that can be developed to a colored state and used to identify the microsphere.

To establish a prima facie case of obviousness, there must be some suggestion or motivation in the reference or in the general knowledge available to one skilled in the art to modify the reference, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all the claim limitations.

**The references fail to disclose a colorless and non-fluorescent molecule:**

As discussed above, Chee et al. describes a colored molecule that changes to a different color or fluoresces. Chee et al. fails to disclose a latent colorant that is colorless and non-fluorescent. As also discussed above, Leblans et al. further fails to teach this limitation. Wang also fails to disclose a colorless and non-fluorescent latent colorant as claimed by the instant invention. Wang makes no reference to colorless and non-fluorescent latent colorants and therefore fails to teach or suggest all of the claimed features of the instant invention. As no reference suggests the use of a color-free, non-fluorescing material that can be switched on to form a colored material, the references fail to provide any suggestion to produce the presently claimed invention. Additionally, claim 13 benefits from its dependency on claim 1, which as discussed above, is patentable.

**The combination of references fail to provide a likelihood of success of solving the problem solved by the instant invention:**

The Examiner fails to provide any motivation for using a non-fluorescing, colorless molecule that can be developed to form a colored material. The references fail to disclose a colorless and non-fluorescent latent colorant as claimed by the instant invention. Furthermore, neither reference discloses any benefit from utilizing non-fluorescent materials or the problems associated with fluorescing materials causing background noise. The present invention deals with the problem of background noise caused by fluorescence, which lowers or interferes with the detectability of a material producing an optical signal. The references fail to address this problem, preferring instead to actually use fluorescent materials.

As discussed above the present invention also provides surprising improvements. The present invention provides improved signal detectability, reduced background noise and a broader dynamic range for measuring target analytes.

Since the references, alone or in combination, fail to suggest the modification necessary to produce the present claims, fail to provide any likelihood of success and fail to disclose all of the present claim limitations, the Applicants request reversal of this rejection.

**Rejection of claims 1, 3, 5 and 6 under 35 U.S.C. § 103(a) over Chee et al. in view of Litt:**

In the Final Office Action dated 04/06/2007 claims 1, 3, 5 and 6 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Chee et al. in view of Litt (US 4,092,408).

The Examiner indicates that the microspheres disclosed by Chee et al. differ from the claimed invention in that the reference does not disclose that the dye is a colorless dye that can be developed to a colored state. The Examiner further indicates that Litt discloses an enzyme label that interacts with colorless o-nitrophenol dyed sugar to produce a measurable color intensity, and that the

enzyme cleaves the sugar from the dye and releases the dye. The intensity of the color is proportional to the enzyme activity. The Examiner states that it would have been obvious to one of ordinary skill in the art to provide the microspheres disclosed by Chee et al. with the dye label disclosed by Litt since the label disclosed by Litt allows the quantification of enzyme activity directly from the intensity of the color produced by the enzyme reaction. This rejection is respectfully traversed.

Chee et al. discloses sensor compositions comprising a composite array of individual arrays, to allow for simultaneous processing of a number of samples. Comprising a substrate with a surface having a plurality of assay locations, each assay location comprising an array location, said array location comprising a plurality of discrete sites; and a population of microspheres comprising at least a first and a second subpopulation, wherein said first subpopulation comprises a first bioactive agent and wherein said second subpopulation comprises a second bioactive agent; wherein said microspheres are distributed in said discrete sites in said array location.

Litt relates to adsorbing Ab<sup>2</sup> from solution in its antiserum and as a solid phase precoating on a substrate followed by adsorbing Ab from its antiserum as a solid phase coating on the Ab<sup>2</sup> coating, followed by carrying out the radioimmunological assay of antigen by contacting the resulting solid phase Ab surface of the double coated substrate with the biological fluid, containing antigen to be assayed and radioactive, fluorescent, or enzyme labeled antigens, to cause the antigen and labeled antigen to become bound in solid phase to the solid phase Ab surface and followed by measuring radioactivity, fluorescence, or enzyme activity of the solid phase bound antigen or of the unbound antigen remaining in solution.

The present invention relates to a microarray comprising a support, on which is disposed a layer of microspheres bearing biological probes, wherein the microspheres comprise at least one material with a non-fluorescent latent colorant that can be developed to a colored state and used to identify the microsphere.

To establish a prima facie case of obviousness, there must be some suggestion or motivation in the reference or in the general knowledge available to

one skilled in the art to modify the reference, there must be a reasonable expectation of success, and the prior art reference must teach or suggest all the claim limitations.

**The references fail to disclose a colorless and non-fluorescent molecule:**

As discussed above, Chee et al. describes a colored molecule that changes to a different color or fluoresces, and fails to disclose a latent colorant that is colorless and non-fluorescent. Litt also fails to disclose this claimed limitation. In fact, Litt teaches the use of fluorescent antigens. (col. 4, lines 14-16). Litt also discloses suitable compounds to include fluorescent particles, and that fluorescent spectrometers may be used to measure the fluorescent labels. (col. 7, lines 41-48). The reference fails to teach any advantages associated with non-fluorescing compounds and instead, suggests that fluorescent compounds are suitable. Neither reference alone or in combination teaches or suggests a colorless and non-fluorescent latent colorant as claimed by the instant invention.

**Litt is non-analogous art:**

Litt discloses an enzyme catalyzed reaction that is incapable of producing a variety of colors. The reference teaches a reaction that is capable of changing color in an assay. The reference fails to disclose any references to microspheres. Furthermore, there is no indication that the dyes disclosed by Litt are compatible with a microarray comprising a layer of microspheres. In fact, the reference discloses a solution which can fluoresce or change to a colored state, but fails to disclose a microsphere containing a latent colorant that can be developed into a colored state to identify the microsphere. The instant invention claims a microarray with a layer of microspheres. Each microsphere is independently capable of changing color. The assay disclosed in the reference is incapable of identifying individual microspheres on a microarray based on the development to a colored state. The reference instead releases dye onto an entire assay, which would result in no distinction between individual microspheres.

**The suggested combination lacks a likelihood of successes:**

The combination of Chee et al. with Litt is inoperable. Chee et al. relates to identifying microspheres in an array. Litt utilizes a dye that can be developed into a colored state in an assay. Utilizing the microspheres disclosed by Chee et al. and the dye disclosed by Litt, it becomes impossible to identify individual microspheres. The dye disclosed by Litt would cover the entire microarray, and once developed into a colored state would create no distinction between the microspheres as taught by Chee et al, making it impossible to identify the individual microspheres. As noted in Col. 7, Lines 48-55 of Litt, it is not the enzyme that changes into a colored state, but the dyed sugar found in the solution. The enzyme cleaves the sugar from the dye thereby releasing the dye into the solution thereby causing the solution to change color.

Neither reference, alone or in combination, teaches all of the claimed limitations of the instant invention. Litt is non-analogous art and the combination of the references is inoperable. Therefore, it is respectfully requested that this rejection be reversed.

**Examiner's response to Applicant's Arguments:**

In the Final Office Action dated 04/06/2007 the Examiner sustains the rejection over claims 1-5 and 7-17 indicating that Leblans et al. discloses the use of colorless photochromic dyes that irreversibly develop into a color once they are developed. However, the reference fails to disclose a dye that is both colorless and non-fluorescent as claimed by the instant invention. Indeed, as discussed above Leblans et al. discloses numerous dyes that fluoresce. Therefore, reversal of the rejections over Chee et al. and Leblans et al. are requested.

In the Final Office Action dated 04/06/2007 the Examiner indicates that the enzyme label disclosed by Litt is not fluorescent. The Examiner states that Litt distinguishes the fluorescent labels from the enzyme labels. However, Litt fails to disclose a colorless, non-fluorescent enzyme label that can be developed into a colored state. As discussed above, the enzyme cleaves sugar from the dye found in solution thereby releasing the dye into the solution. Releasing the dye

into solution develops the solution into a colored state, not the enzyme. Therefore, the reference fails to teach this claimed limitation.

The Examiner further indicates that the it is unclear why the dye disclosed by Litt must cover the entire microarray and that an assay can comprise a single bioactive agent making the ability to distinctly identify individual microspheres unnecessary.

Regarding the dye of Litt covering the entire microarray, as discussed above Litt does not disclose microspheres. The reference discloses an enzyme that cleaves sugar from dye to release the dye into the solution. The entire solution then changes color. Once injected into a microarray the dye would coat the entire microarray eliminating the ability to identify individual microspheres as claimed by the instant invention.

Regarding making it unnecessary to distinctly identify individual microspheres, the instant invention claims a layer microspheres having a colorless and non-fluorescent latent colorant that can be developed to a colored state and used to identify said microsphere. The instant invention claims a plurality of microspheres each capable of being identified by development to a colored state. As discussed above coloring the entire microarray defeats this objective.

### **Summary**


The microsphere of the present invention overcome the problem associated with “spectrally addressed microspheres,” colored compounds typically used in microspheres are often fluorescent, and hence provide excessive background noise when fluorimetric determinations are performed on the microarray. This problem is overcome through the use of latent colorants, which are colorless and non-fluorescent until switched to a colored state by a chemical reaction, a physical trigger, or some kind of environmental stimulus. A further surprising result of the present invention is that the colorless coding offers no detectable background fluorescence from microspheres, therefore detection is dramatically improved. As such, the dynamic range for measuring target analytes is greatly increased over the prior art.

Chee et al. discloses a colored molecule that changes to a different color or fluoresces. Chee et al. fails to disclose a molecule that is both colorless and non-fluorescent as claimed by the instant invention. Leblans et al. also fails to disclose a colorless non-fluorescent colorant. Indeed, Leblans et al. discloses the use of fluorescent materials throughout the reference. Neither Wang nor Litt make any reference to colorless and non-fluorescent latent colorants. In fact, Litt teaches the use of fluorescent antigens. Furthermore, Litt discloses a dye, that once injected into a microarray, prevents the individual identification of microspheres.

### **Conclusion**

For the above reasons, Appellants respectfully request that the Board of Patent Appeals and Interferences reverse the rejection by the Examiner and mandate the allowance of Claims .

Respectfully submitted,

  
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Enclosures

## **Appendix I - Claims on Appeal**

1. A microarray comprising:  
a support; on which is disposed;  
a layer of microspheres bearing biological probes; wherein said microspheres comprise at least one colorless and non-fluorescent latent colorant that can be developed to a colored state and used to identify said microsphere.
2. The microarray of claim 1 wherein the microspheres are arranged on the support in random distribution.
3. The microarray of claim 1 wherein the latent colorant is capable of being developed to an optical signature.
4. The microarray of claim 3 wherein the optical signature is absorbance.
5. The microarray of claim 3 wherein the latent colorant is capable of being developed to an optical signature by chemical or physical means.
6. The microarray of claim 5 wherein the chemical means is condensation reaction, acid-base reaction, redox reaction, abstraction reaction, addition reaction, elimination reaction, concerted reaction, chain propagated



reaction, complexation reaction, molecular coupling reaction, rearrangement, or a combination of two or more of the foregoing.

7. The microarray of claim 5 wherein the physical means is a photo initiated process, a thermo initiated process, an ionizing radiation initiated process, an electron beam initiated process, an electrical initiated process, a pressure initiated process, a magnetic initiated process, an ultrasound initiated or a combination of two or more of the foregoing.

8. The microarray of claim 3 wherein the optical signature can be used to identify a target analyte.

9. The microarray of claim 1 wherein the material with a latent color is a leuco dye, a precursor of a leuco dye, a photographic coupler, a metal complexing ligand, a photochromic dye, or a thermochromic dye.

10. The microarray of claim 1 wherein the biological probe is bioactive.

11. The microarray of claim 10 wherein the bioactive probe comprises polynucleotide, polypeptide, polysaccharides, or small synthetic molecules.

12. The microarray of claim 1 wherein the microspheres are immobilized on a two dimensional support by chemical or physical interactions.

13. The microarray of claim 1 wherein the microspheres are immobilized on a two dimensional support by a gelation process.

14. The microarray of claim 1 wherein the microspheres have a mean diameter of 1 to 50 microns.

15. The microarray of claim 1 wherein the microspheres have a mean diameter of 5 to 20 microns.

16. The microarray of claim 1 wherein the concentration of microspheres on the support is 100 to a million per  $\text{cm}^2$ .

17. The microarray of claim 1 wherein the concentration of microspheres on the support is 10,000 to 100,000 per  $\text{cm}^2$ .

## **Appendix II - Evidence**

None.

### **Appendix III – Related Proceedings**

None.